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Spartina alterniflora invasion alters soil bacterial communities and enhances soil N_2O emissions by stimulating soil denitrification in mangrove wetland

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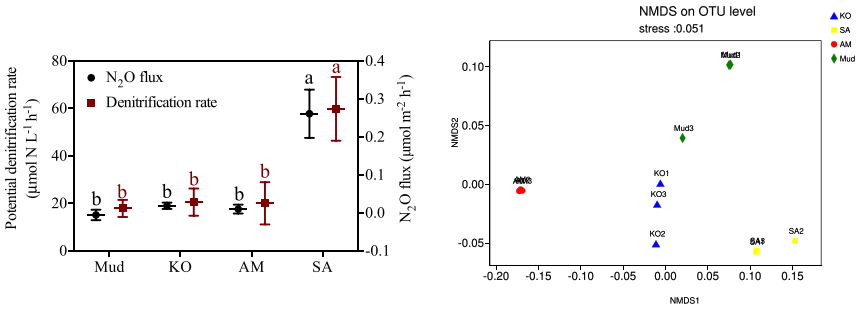
HIGHLIGHTS

- *S. alterniflora* invasion significantly ($p < 0.05$) increased soil N_2O emissions.
- *S. alterniflora* invasion significantly ($p < 0.05$) enhanced soil denitrification.
- *S. alterniflora* invasion decreased bacterial α-diversity and strongly modified soil bacterial community.
- Indicator species *Bacilli*, *Alphaproteobacteria*, and *Chloroflexia* were specially associated with *S. alterniflora*.
- Soil organic matter (SOM) and pH were key environmental factors in altering soil bacterial community.

GRAPHICAL ABSTRACT



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ABSTRACT

Chinese mangrove, an important ecosystem in coastal wetlands, is sensitive to the invasive alien species *Spartina alterniflora*. However, the effects of the *S. alterniflora* invasion on mangrove soil N_2O emissions and the underlying mechanisms by which emissions are affected have not been well studied. In this study, the N_2O emitted from soils dominated by two typical native mangroves (i.e. *Kandelia obovata*: KO; *Avicennia marina*: AM), one invaded by *S. alterniflora* (SA), and one bare mudflat (Mud) were monitored at Zhangjiang Mangrove Estuary (where *S. alterniflora* is exotic). Together with soil biogeochemical properties, the potential denitrification rate and the composition of soil bacterial communities were determined simultaneously by $^{15}NO_3^-$ tracer and high-throughput sequencing techniques, respectively. Our results showed that *S. alterniflora* invasion significantly ($p < 0.05$) increases soil N_2O emissions by 15–28-fold. In addition, isotope results revealed that the soil potential denitrification rate was significantly ($p < 0.05$) enhanced after *S. alterniflora* invasion. Moreover, the *S. alterniflora* invasion significantly ($p < 0.05$) decreased soil bacterial α-diversity and strongly modified soil bacterial communities. Indicator groups strongly associated with *S. alterniflora* were *Chloroflexia*, *Alphaproteobacteria*, and *Bacilli*, each of which was abundant and acts as connector in the co-occurrence network. FAPROTAX analysis implied that the *S. alterniflora* invasion stimulated soil denitrification and nitrification while depressing anaerobic

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ammonium oxidation (anammox) and dissimilatory nitrate reduction to ammonium (DNRA). Redundancy analysis (RDA) found that soil organic matter (SOM) and pH were the most important environmental factors in altering soil bacterial communities. Taken together, our results imply that the *S. alterniflora* invasion in mangrove wetlands significantly stimulates soil denitrification and N_2O emissions, thereby contributing N_2O to the atmosphere and contributing to global climate change.

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1. Introduction

Nitrous oxide (N_2O) is an important long-lived greenhouse gas that contributes to global warming and is involved in atmospheric ozone depletion (IPCC, 2013). As an important intermediate product of soil nitrification and denitrification processes, N_2O emissions are closely linked to soil microbe activity (Butterbach-Bahl et al., 2013). It is estimated that >80% of global N_2O emissions can be attributed to soil microbial activity (IPCC, 2013). Both soil aerobic nitrification and anaerobic denitrification are fundamental processes of N_2O production and consumption in terrestrial ecosystems (Butterbach-Bahl et al., 2013). However, soil denitrification is responsible for a large proportion of N_2O production in tropical and subtropical mangroves, by some estimates accounting for as much as 43–93% of total N_2O production (Chiu et al., 2004; Fernandes et al., 2010).

Mangrove, one of the most important ecosystems in tropical and subtropical coastal wetlands, provides many valuable ecosystem services (Duke et al., 2007). However, Chinese mangrove ecosystems are vulnerable to the invasive species *Spartina alterniflora*, which was introduced in the 1970s and has spread throughout mangrove coastlines over the past few decades (Zhang et al., 2017). Zhangjiang Mangrove Estuary, the present study site, was invaded by *S. alterniflora* in the 1990s. Over the past decade, the total area occupied by *S. alterniflora* has rapidly expanded from 57.94 ha to 116.11 ha (Liu et al., 2017a).

Extensive studies have been conducted to investigate the effects of *S. alterniflora* invasion on soil N_2O emissions (Chen et al., 2015; Yin et al., 2015; Yuan et al., 2015). However, to date the effect of the *S. alterniflora* invasion on wetland soil N_2O emissions remains controversial. The *S. alterniflora* invasion was reported to decrease the soil N_2O emissions of *Suaeda salsa* and *Phragmites australis* salt marshes along the eastern coast of China (Yin et al., 2015; Yuan et al., 2015). However, in mangrove wetlands in the Jiulong River Estuary, Chen et al. (2015) reported that soil N_2O emissions from sites containing *S. alterniflora* were higher than the emissions from mangrove sites containing *Kandelia obovata*, *Sonneratia apetala*, or *Cyperus malaccensis*. In addition, Wang et al. (2016b) found no significant differences in soil N_2O emissions of *S. alterniflora* and *K. obovata* soils in the Jiulong River Estuary. These studies mainly focused on the relationships between soil N_2O emissions and soil biogeochemical properties. However, there exists little data regarding the effects of the *S. alterniflora* invasion on the soil denitrification rate or the soil bacterial community structure and composition in mangrove wetlands.

Recently, a meta-analysis conducted by Alldred and Baines (2016) found that denitrification activity was higher at *S. alterniflora* sites than at sites dominated by other plant species. However, a recent study found no significant differences in soil denitrification in a *S. alterniflora* salt marsh and a mudflat at Sapelo Island, Georgia (He et al., 2016). In addition, Liu et al. (2017b) found that the *S. alterniflora* invasion strongly modified the soil microbial community structure and composition in a mangrove wetland in the Jiulong River Estuary. Taken together, the relationship between soil N_2O emissions, soil denitrification rates, and soil microbes remains unclear.

Previous studies in terrestrial ecosystems demonstrated that changes in the prevalence of aboveground plant species plays an important role in soil N dynamics (Wardle et al., 1994), N pool size (Ehrenfeld, 2003), and the composition of soil microorganism communities (Sasse et al., 2018). Therefore, we assumed that the geographic expansion of *S. alterniflora* may have a great impact on soil microbial community structure, soil denitrification rate, and soil N_2O emissions in coastal mangrove wetlands. To test our hypothesis, a field study was performed to investigate whether differences in soil N_2O emissions, bacterial community composition, and denitrification rates exist between *S. alterniflora* and native mangrove sites.

2. Materials and methods

2.1. Study site

The current study was carried out in Zhangjiang River Estuary Mangrove National Natural Reserve (23°55′N, 117°26′E) in Yunxiao County, Fujian Province, China (Fig. 1). This area is subject to subtropical marine climate with a mean annual air temperature of 21.5 °C. In this intertidal zone, *S. alterniflora* has rapidly spread since 2005 and been widely distributed along with native mangrove wetland which were mainly dominated by mangrove species *K. obovata* and *Avicennia marina* (Liu et al., 2017a). This area is experiencing irregular semidiurnal tide with an average range of 2.32 m (Zhang et al., 2006). *S. alterniflora* was mainly expanding in bare mudflat in this area (Liu et al., 2017a). Hence, bare mudflat (Mud) site was selected as the habitats before *S. alterniflora* invasion. In addition, soil occupied by two typical mangrove species (*K. obovata*: KO and *A. marina*: AM) and one invasive species (*S. alterniflora*: SA) were randomly established with three replicates (Fig. 1). The species abundances in a 10 * 10 m plot of *K. obovata*, *A. marina*, and *S. alterniflora* were >95% at KO, AM, and SA sites, respectively. All sites are at least 20 m away from each other to avoid the edge effects, while experiencing similar tidal dynamic and exposure time.

2.2. N_2O collection and quantification

Soil-atmosphere exchanges of N_2O were collected in 2016 (Jul, Aug and Sep) and 2017 (Jun, Jul and Aug). Soil N_2O emissions were measured using traditional closed chamber couple with gas chromatography (Chen et al., 2012a). The chamber has an internal volume of 2 L and an enclosed area of 0.03 m^2, similar to Chen et al. (2012a). Gas samples were collected during the neap tide between 12:00–14:00 local time. Sampling procedures were performed according to Gao et al. (2018). Chamber air was mixed carefully and 10 ml gas was collected by a glass syringe at 0, 5, 10 and 15 min after closure. Gas samples were then injected into pre-evacuated fluorinated ethylene propylene (FEP) Teflon air bags and analyzed within 24 h. Air temperature inside the chamber was simultaneously measured.

N_2O concentrations were quantified by gas chromatograph (Agilent 7890B, CA, USA) equipped with a ^{63}Ni electron capture detector (ECD). To ensure data quality and stability, gradient standard gases (purchased from the National Institute of Metrology, China) were inserted into the GC system every hour. Soil N_2O emissions were calculated using a linear least squares to fit the gradient standard curves with time series. Data were accepted if the linear fitting of $R^2 > 0.90$. In total, twenty-two data set were generated initially and twenty data set were accepted for further calculating.

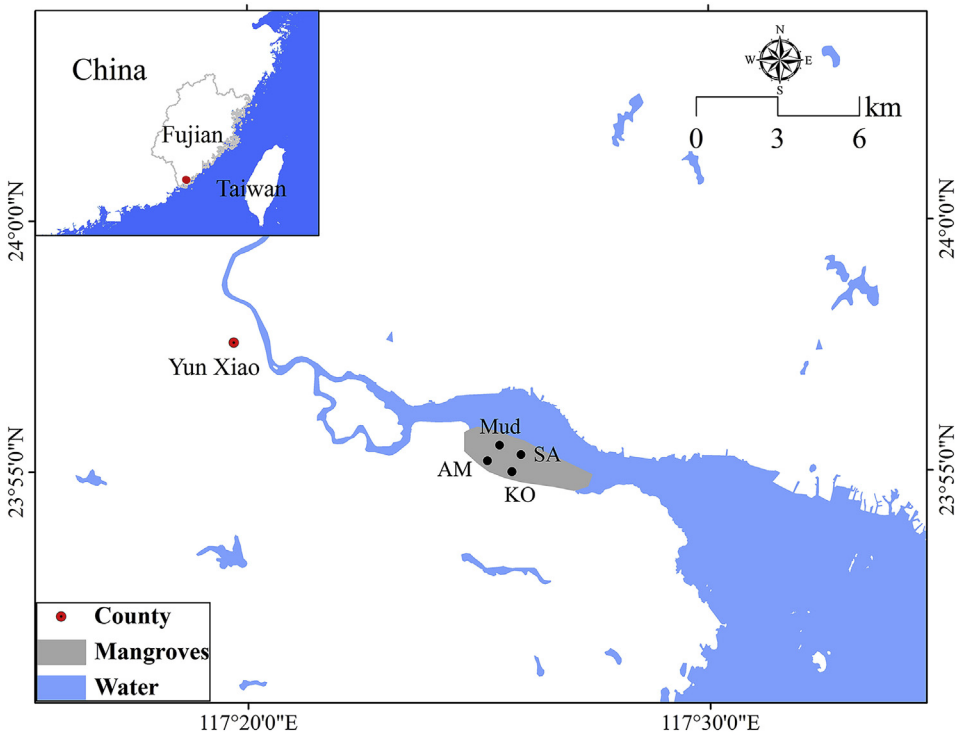

Fig. 1. Location of the Zhangjiang River Estuary Mangrove National Natural Reserve and our sampling sites. KO: *K. obovata*; AM: *A. marina*; SA: *S. alterniflora*; Mud: bare mudflat.

2.3. Soil biogeochemical properties measurements

Intact soil cores (0–20 cm in depth) were randomly collected from each site using a PVC tube (6 cm in diameter) in Aug 2016 and Jul 2017, respectively. Soil cores were sealed immediately with gummed tape and stored on ice bags during the transportation. In laboratory, each soil core was completely homogenized after removing the plant roots and other debris. Salinity and pH of pore-water (collected from field sites) were determined using an Orion 3 star digital portable pH meter (Thermo, USA) and MASTER-S/MillM salinity refractometer (ATAGO, Japan), respectively. Water content was measured by oven-drying of 50 g fresh soil to a constant weight at 30 °C (Buchmann, 2000). The dried soil was then ground into powder and sieved through 2 mm mesh sieves. Organic matter content (SOM) was determined based on the loss on ignition at 550 °C for 6 h after 105 °C oven-dried (Heiri et al., 2001). Total carbon (TC), total organic carbon (TOC), total nitrogen (TN) and C/N ratio were measured using Vario EL III Elemental Analyzer (Elementar, Hanau, Germany). Before TOC determination, soils were decarbonized using 1 mol/L HCl (Zhang et al., 2010). A 10 g fresh soil was extracted with 50 ml 2 M KCl and filtered through a 0.45 μm membrane filters after shaken at 200 rpm for 1 h (Chen et al., 2012a). The extracts were subsequently analyzed for inorganic N (NH_4^+, NO_3^- and NO_2^-) concentration using AA3 Auto Analyzer 3 (Seal, Germany). Soil urease, nitrate reductase and nitrite reductase were measured according to the manufacturer instructions using Solid-Urease Kit (detect limits: 0.07 μmol/d/g DW), S-NR Test Kit (detect limits: 0.01 μmol/d/g DW) and SNIR Kit (detect limits: 0.05 μmol/d/g DW), respectively, which were purchased from Suzhou Comin Biotechnology Co., Ltd. (Suzhou, China).

2.4. Soil potential denitrification rate measurements using ^{15}N isotope technology

Soil samples collected in Jul 2017 were applied to determine the soil potential denitrification rate via slurry incubations (Hsu and Kao, 2013). In brief, soil in each core was homogenized with in situ seawater in a ratio of 1:1 (v/v). The slurry was well mixed and purged with pure helium gas (>99.999%) for 1 h. 3 ml premixed anoxic slurry was then moved into 12 ml gas-tight vials (Exetainers) and flushed with pure helium gas for 2 min. Above procedures were performed in an oxygen-free glove bag. All samples were incubated for 20 h to consume residual oxygen at 25 °C. $^{15}NO_3^-$ tracer (Sigma-Aldrich, 98 ^{15}N atom %) was added to the slurry to a final concentration of 100 μM and incubated for 0, 1, 2, 3 h. Samples were fixed with 100 μl 7 M $ZnCl_2$ solutions at the end of the incubation. All fixed samples were then kept upside down at room temperature in darkness before analysis.

The concentration of $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ in fixed samples headspace air was analyzed using an isotope ratio mass spectrometer (Delta V Advantage, Thermo Finnigan, California, USA). In the present study, $^{45}N_2O$ and $^{46}N_2O$ concentrations were not determined as assuming that the complete denitrification process occurred during the incubations (Crowe et al., 2012). Soil potential denitrification rates were calculated based on the $^{29}N_2$ (D_{29}) and $^{30}N_2$ (D_{30}) production, according to Tan et al. (2017).

$$D_{potential} = D_{14} + D_{15} = \left(\frac{D_{29}}{2 \times D_{30}} + 1\right) \times (D_{29} + 2 \times D_{30})$$

2.5. Soil DNA extraction and sequencing libraries constructions

Soil obtained in Jul 2017 was used for high-throughput sequencing. Soil genome DNA was extracted using the FastDNA SPIN Kit for Soil (MP, CA, USA) according to the manufacturer instructions. DNA quality and quantity were checked using a Nanodrop ND-1000 spectrophotometer (NanoDrop Tech, Wilmington, USA) and 1% agarose gels electrophoresis. 16S V4-V5 regions of 16S rRNA gene were amplified using the primer pairs of 515F and 907R with the barcode (Supporting Information Table 1, Supporting Information Table 2). PCR products were monitored on 2% agarose gels electrophoresis and purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were constructed using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) and index codes were added. The library quality was

assessed on Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on Illumina HiSeq2500 platform (Novogene, Beijing, China) and 250 bp paired-end reads were generated.

2.6. Illumina sequencing data analyses

Paired-end reads was assigned to samples based on their unique barcode and truncated and merged using FLASH (V1.2.7) (Magoč and Salzberg, 2011). High-quality clean tags were generated under specific filtering conditions according to the QIIME (V1.7.0) quality controlled process (Caporaso et al., 2010; Bokulich et al., 2013). Effective tags were obtained after removing the chimera sequences using UCHIME algorithm (Edgar et al., 2011). Sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs) by Uparse software (Uparse v7.0.1001) and annotated using GreenGene Database based on RDP classifier (Version 2.2) after removing singletons (DeSantis et al., 2006; Edgar, 2013). OTUs abundance information was normalized using a standard of the sequence number corresponding to the sample with the least sequences. Subsequent analysis of α- and β- diversity was performed based on this normalized data through the free online platform of I-Sanger (http://www.i-sanger.com).

Community richness index (Sobs, Chao and ACE) and community diversity index (Simpson and Shannon) were used to estimate α-diversity and examined by Welch's *t*-test. Relative abundance of top 10 phyla was displayed by barplot. In addition, a Venn Diagram was used to show the share and unique genera. For β-diversity analysis, the hierarchical clustering tree based on Weighted-UniFrac distance and the non-metric multi-dimensional scaling (NMDS) analysis based on Bray-Curtis distance were performed on OTU level.

Variance inflation factor (VIF) analysis was used to test the severity of multicollinearity of environmental factors. Environmental factors with VIF > 10 were removed. Redundancy analysis (RDA) was conducted to explore the most important factors in influencing soil bacterial communities on OTU level (R 3.4.1). In addition, the Spearman correlations between the relative abundances of top 20 classes and the key variables were showed.

Indicator species analysis was conducted to identify the classes that were specially associated with each site (Dufrêne and Legendre, 1997). Indicator analysis was performed using 'labdsv' package and visualized using R (R 3.4.1).

The co-occurrence network was constructed using Spearman correlation which calculated by 'WGCNA' package (Langfelder and Horvath, 2012) (R 3.4.1). Genera with relative abundance <0.01% were deleted. All *p*-values were adjusted by Benjamini and Hochberg false discovery rate (FDR) using 'multtest' package (Benjamini et al., 2006) (R 3.4.1). Co-occurrence network was visualized using Gephi (0.9.2, http://gephi.github.io/) by setting the *p*-values and correlation coefficient of 0.05 and 0.70 cutoff, respectively. The network modularity and modularity roles were determined using simulated annealing by 'rnetcarto' package (R 3.4.1).

Functional Annotation of Prokaryotic Taxa (FAPROTAX), a database that extrapolates functions of cultured prokaryotes to estimate metabolic or other ecological relevant functions, was used to evaluate the N dynamics after *S. alterniflora* invasion (Louca et al., 2016).

2.7. Statistical analyses

Normality and homogeneity of variance of all data were tested using Shapiro-Wilk normality and Bartlett test, respectively (R 3.4.1). Ordinary one-way ANOVA followed by Tukey's multiple comparison tests were used to compare the significant difference of soil N_2O emissions, soil biogeochemical properties, α-diversity indexes and soil potential denitrification rates (SPSS, 22.0). The similarity between and within groups was estimated by the Analysis of Similarities (ANOSIM) and Multi Response Permutation Procedure (MRPP) tests based on Bray-Curtis distance (R 3.4.1).

3. Results

3.1. Soil N_2O emissions

Soil N_2O emissions exhibited significant (F-statistic = 16.91, $p < 0.001$) differences among sites (Fig. 2). Our results showed that soil N_2O emissions at the Mud site ranged from −0.32 to 0.19 μmol m^{-2} h^{-1} with a mean emission of −0.01 μmol m^{-2} h^{-1}. At the KO and AM sites, the average soil N_2O emissions were 0.02 and 0.01 μmol m^{-2} h^{-1}, respectively. No significant ($p > 0.05$) differences were found among the Mud, KO, and AM sites. However, the soil N_2O emitted from the SA site reached 0.26 μmol m^{-2} h^{-1}, which was significantly ($p < 0.05$) higher than the amounts released at the other three sites.

3.2. Soil biogeochemical properties

We found that soil biogeochemical properties exhibited significant ($p < 0.05$) spatial heterogeneity (Table 1). The mangrove soils of the KO and AM sites were slightly acidic, whereas the SA and Mud sites were marginally alkaline. The observed pH at the SA site was significantly ($p < 0.005$) higher than that at the KO and AM sites. We found that salinity and water content showed a similar trend, with both variables following a SA > AM > KO > Mud trend among sites. The presence of plant vegetation (AM, KO, and SA) was associated with higher soil organic matter content and TOC, however, the AM, KO, and SA sites did not show statistically significant (i.e. $p > 0.05$). The highest and lowest NO_3^- concentrations were found at the SA and AM sites, respectively ($p < 0.05$). In addition, the SA site was found to have the highest ($p < 0.05$) TN value, nitrate reductase activity, and nitrite reductase activity. Furthermore, the highest TC and C/N ratios were found at the AM site ($p < 0.05$), while no significant ($p > 0.05$) differences were found among sites for NH_4^+, NO_2^- concentration, and urease activity.

3.3. Soil potential denitrification rate

$^{15}NO_3^-$ isotope results showed that the highest ($p < 0.05$) soil potential denitrification rate was observed at the SA site (Fig. 3, Supporting Information Fig. 1). We found that the soil potential denitrification rate reached 59.79, 19.98, 20.50, and 17.83 μmol N l^{-1} h^{-1} at the SA, AM, KO, and Mud sites, respectively. The soil potential denitrification rate at the SA site was about 2.92–3.35 times higher than the rates found at the other three sites. In addition, no significant ($p > 0.05$) differences

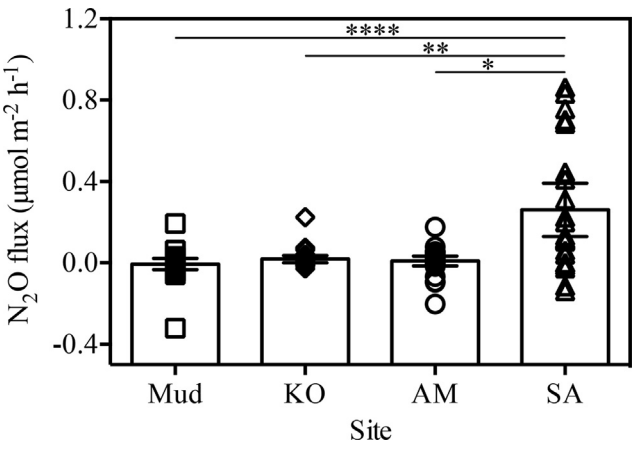

Fig. 2. Spatial differences of N_2O emission (μmol m^{-2} h^{-1}). Mud: bare mudflat, marked as a square; KO: *K. obovata*, marked as diamond-shaped; AM: *A. marina*, marked as a circle; SA: *S. alterniflora*, marked as a triangle. *means $p < 0.05$; **means $p < 0.01$; ****indicates $p < 0.001$.

Table 1
Spatial difference in soil biogeochemical properties. DW: dry weight. Data was given in 20 cm depth and expressed as mean ± SE. Different lowercase letters indicate significant difference at $p < 0.05$. KO: *K. obovata*; AM: *A. marina*; SA: *S. alterniflora*; Mud: bare mudflat.

Biogeochemical properties	Mud	KO	AM	SA
pH	7.34 ± 0.04 **a**	6.95 ± 0.05 **b**	6.83 ± 0.06 **b**	7.28 ± 0.02 **a**
Salinity	9.27 ± 0.02 **d**	10.97 ± 0.09 **c**	11.53 ± 0.15 **b**	14.53 ± 0.15 **a**
Water content (%)	39.39 ± 0.19 **d**	42.85 ± 0.15 **c**	46.96 ± 0.06 **b**	50.47 ± 1.30 **a**
Soil organic matter (%)	15.99 ± 0.28 **c**	18.05 ± 0.24 **b**	20.83 ± 0.12 **a**	18.12 ± 0.15 **b**
NH_4^+ concentration (μmol/g DW)	0.83 ± 0.01 **a**	0.84 ± 0.10 **a**	0.95 ± 0.11 **a**	0.91 ± 0.07 **a**
NO_3^- concentration (nmol/g DW)	23.87 ± 2.10 **b**	19.19 ± 0.32 **bc**	17.42 ± 1.29 **c**	32.90 ± 1.29 **a**
NO_2^- concentration (nmol/g DW)	0.43 ± 0.22 **a**	0.22 ± 0.04 **a**	0.22 ± 0.03 **a**	0.22 ± 0.04 **a**
TC (mg/g DW)	9.28 ± 1.02 **b**	11.43 ± 1.21 **ab**	15.01 ± 0.92 **a**	11.19 ± 0.22 **ab**
TOC (mg/g DW)	8.12 ± 0.13 **b**	10.66 ± 0.11 **a**	10.85 ± 0.52 **a**	10.21 ± 0.19 **a**
TN (mg/g DW)	1.00 ± 0.00 **b**	1.17 ± 0.03 **ab**	1.17 ± 0.09 **ab**	1.23 ± 0.03 **a**
C/N ratio	9.03 ± 0.30 **c**	11.37 ± 0.04 **ab**	11.79 ± 0.75 **a**	9.90 ± 0.07 **bc**
Urease (μmol/d/g DW)	52.76 ± 4.57 **a**	56.75 ± 2.63 **a**	70.96 ± 5.73 **a**	59.19 ± 8.43 **a**
Nitrate reductase (μmol/d/g DW)	3.68 ± 0.58 **b**	5.08 ± 0.21 **b**	5.66 ± 0.40 **ab**	7.78 ± 0.88 **a**
Nitrite reductase (μmol/d/g DW)	5.48 ± 0.71 **b**	5.64 ± 0.72 **b**	6.11 ± 0.98 **ab**	9.73 ± 1.01 **a**

in soil potential denitrification rate were found among the AM, KO, and Mud sites.

3.4. Soil bacterial community composition and structure

Results of an α-diversity analysis showed that the SA site had the lowest ($p < 0.05$) ACE species richness and Shannon index scores, while this site also had a higher ($p < 0.05$) Simpson index relative to the other three sites (Table 2). The 5 most abundant bacteria at all sites were *Deltaproteobacteria*, *Gammaproteobacteria*, *Chloroflexi*, *Alphaproteobacteria*, and *Acidobacteria*. These five clades accounting for >60% of all identified bacteria (Fig. 4a). A higher relative abundance of *Gammaproteobacteria* and *Alphaproteobacteria* was observed at the SA site (Fig. 4a). At the genus level, Venn Diagram analysis showed that the bacterial composition of the SA site was distinct from each of the other sites (Fig. 4b). Although all sites had representative bacteria from most genera, 20 unique genera were observed at the SA site (Fig. 4b). The results of hierarchical clustering tree and NMDS (stress = 0.05) analyses revealed that the bacterial communities found at the SA site were different from those collected at the other sites (Fig. 4c, d). MRPP and ANOSIM also showed a *A*-statistic value of 0.39 ($p < 0.001$) and a statistic values of 1.00 ($p < 0.001$), respectively, indicating that the soil bacterial community was significant different from each other (Table 3, Supporting Information Table 3).

3.5. The relationship between soil biogeochemical properties and the soil bacterial community

In order to explore factors affecting soil bacterial communities, RDA was performed after evaluation by VIF. Our results showed that SOM and pH were the most important factors regulating the soil bacterial community composition (Fig. 5). Axes 1 and 2 were able to explain 44.41% and 12.81% of the total variations, respectively. A Spearman correlation analysis showed that the relative abundance of *Alphaproteobacteria*, *Gemmatimonadetes*, and *Planctomycetacia* was significantly ($p < 0.05$) positively correlated with pH, while the relative abundance of *Deltaproteobacteria* and *Bacteroidia* was significantly ($p < 0.05$) negatively correlated with pH (Supporting Information Fig. 2). In addition, the relative abundance of *Bacteroidia* was also found to be significantly ($p < 0.05$) positively correlated with SOM. However, the relative abundance of *Flavobacteriia*, *norank_p_Latescibacteria*, *Sphingobacteriia*, and *Gemmatimonadetes* was significantly ($p < 0.05$) negatively correlated with SOM.

3.6. Indicator species that were specially associated with S. alterniflora and the co-occurrence network

We performed an indicator species analysis to identify specific species that were strongly associated with each site (Fig. 6, Supporting Information Table 4). The results of this analysis showed that the

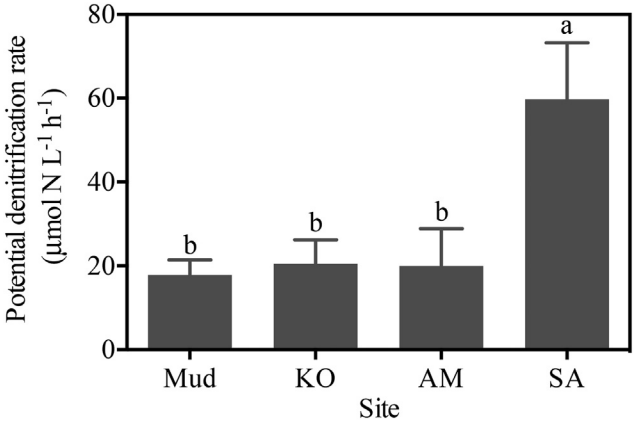

Fig. 3. Soil potential denitrification rates (μmol N L^{-1} h^{-1}) based on ^{15}N isotope technology. KO: *K. obovata*; AM: *A. marina*; SA: *S. alterniflora*; Mud: bare mudflat. Different lowercase letters indicate significant differences in mean value at $p < 0.05$.

Table 2
α-diversity estimates at genus level. Different lowercase letters indicate significant difference at $p < 0.05$ by Welch's *t*-test. KO: *K. obovata*; AM: *A. marina*; SA: *S. alterniflora*; Mud: bare mudflat.

Sample name	Sobs	Chao	ACE	Shannon	Simpson
Mud1	1015	1133.309	1126.595	5.150	0.014
Mud2	1004	1124.161	1109.504	5.131	0.014
Mud3	1040	1151.781	1146.689	5.221	0.013
KO1	1025	1136.029	1139.514	5.235	0.013
KO2	1028	1145.102	1141.583	5.270	0.012
KO3	1042	1143.753	1138.758	5.267	0.012
AM1	993	1112.658	1088.037	5.232	0.012
AM2	990	1076.443	1079.743	5.219	0.012
AM3	1031	1136.010	1142.135	5.240	0.012
SA1	1042	1139.146	1130.425	5.184	0.015
SA2	994	1132.734	1121.048	5.142	0.015
SA3	1017	1147.172	1124.849	5.157	0.014
Mud	1019.7 **a**	1136.4 **a**	1127.6 **ab**	5.167 **ab**	0.014 **a**
KO	1031.7 **a**	1141.6 **a**	1140.0 **a**	5.257 **a**	0.012 **b**
AM	1004.7 **a**	1108.4 **a**	1103.3 **ab**	5.230 **a**	0.012 **b**
SA	1017.7 **a**	1139.7 **a**	1125.4 **b**	5.161 **b**	0.015 **a**

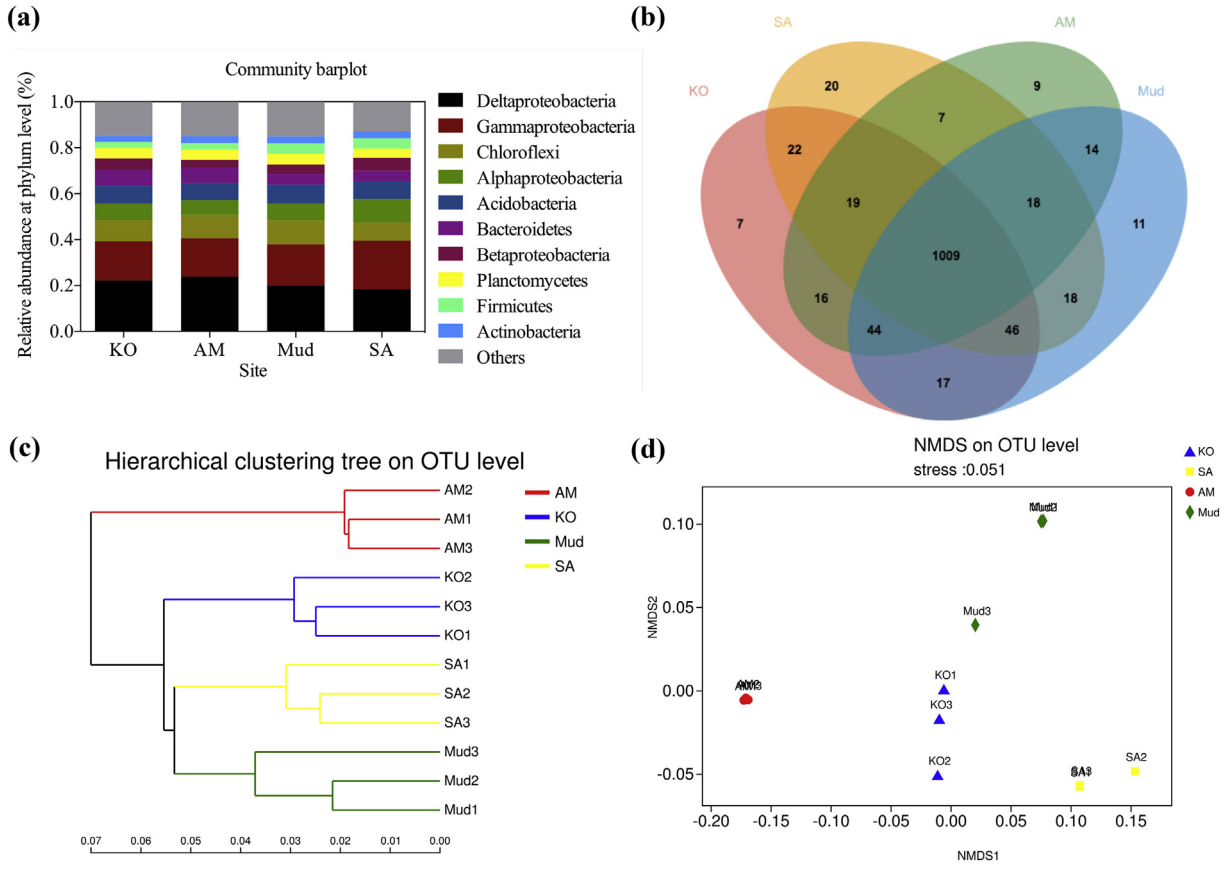

Fig. 4. Differences in soil bacterial communities among sites. Relative abundance of the top 10 phyla (a); Venn plot at genus level (b); hierarchical clustering tree on OTU level using Weighted-UniFrac distance (c); non-metric multi-dimensional scaling (NMDS) on OTU level using Bray-Curtis distance (d). KO: *K. obovata*; AM: *A. marina*; SA: *S. alterniflora*; Mud: bare mudflat.

most abundant indicator classes were varied among sites. The most abundant indicators were *Acidimicrobiia*, *Deltaproteobacteria*, *Ignavibacteria*, and *Alphaproteobacteria* at the Mud (1.38%), AM (23.91%), KO (1.39%), and SA (10.31%) sites, respectively. In addition, *Bacilli* and *Chloroflexia* were also specially associated with the SA site, and accounted for 0.81% and 0.11% of total bacterial abundance, respectively.

A co-occurrence network was constructed where genera were plotted as nodes and the correlations between genera were plotted as edges. The co-occurrence network consisted of 186 nodes and 1307 edges, and 81.87% of the resulting correlations were positive (Fig. 7a, Supporting Information Table 5). Indicators specially associated with the SA site included *Chloroflexia*, *Bacilli*, and *Alphaproteobacteria*, which belong to the *Chloroflexi*, *Firmicutes*, and *Alphaproteobacteria*, respectively (Fig. 6). *Chloroflexi* (2.69%), *Firmicutes* (9.68%), and *Alphaproteobacteria* (13.98%) were abundant and act as connectors in the co-occurrence network, which suggests that the *S. alterniflora* invasion may have had a strong impact on soil bacterial communities (Fig. 7b).

Table 3
Results of ANOSIM and MRPP test in bacterial community composition among different sites.

	ANOSIM	MRPP
Statistic	1.000	
A statistic		0.393
p value	0.001	0.001
Permutation number	999	999

3.7. FAPROTAX analysis reveals that the S. alterniflora invasion has strongly affected soil N dynamics

FAPROTAX analysis was used to assess the impact of the *S. alterniflora* invasion on soil N processes (Fig. 8). Similar to the results of the $^{15}NO_3^-$ tracer experiment, FAPROTAX analysis showed the highest degree of denitrification at the SA site (Fig. 3, Fig. 8). A relatively higher level of N fixation was also found at SA the site, indicating that

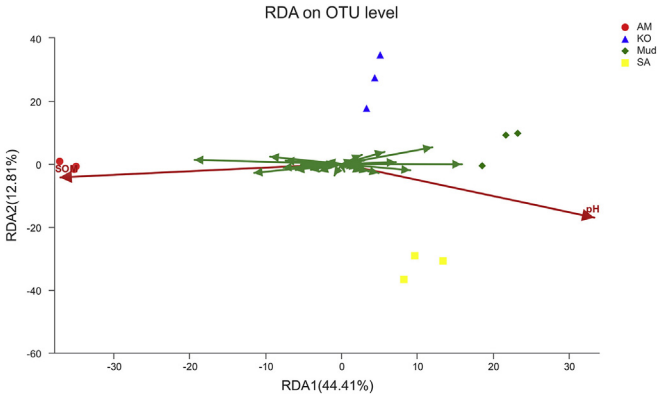

Fig. 5. Redundancy analysis (RDA) of soil biogeochemical properties on soil bacterial communities. Environmental factors were chosen by using a Variance inflation factor (VIF) analysis with VIF < 10. TN: total nitrogen; SOM: soil organic matter. KO: *K. obovata*; AM: *A. marina*; SA: *S. alterniflora*; Mud: bare mudflat. Green arrows indicated OTUs with a top 30 relative abundance.

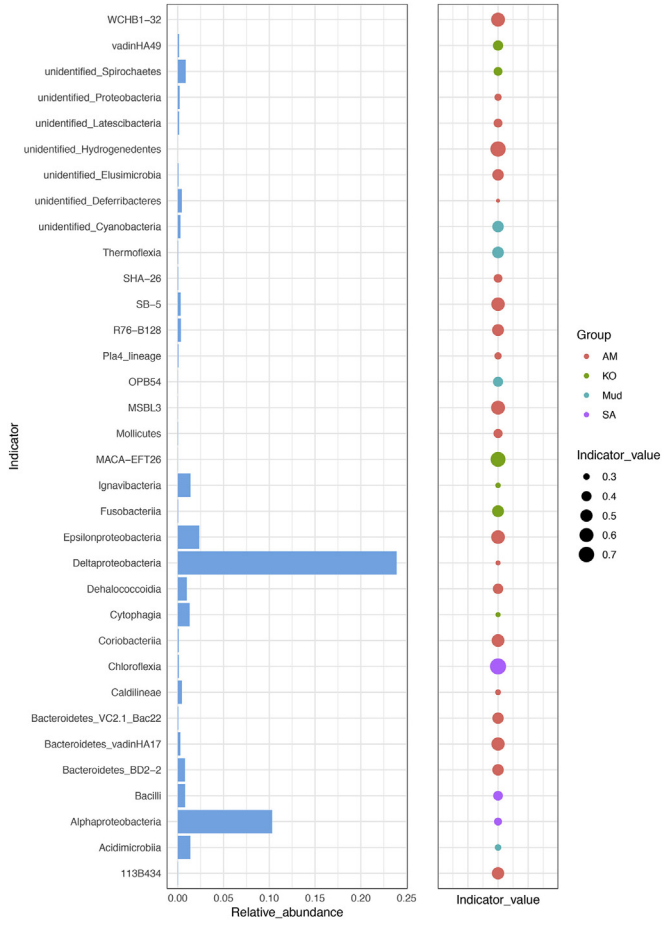

Fig. 6. Indicator species that specially associated with each site. Columns represents the relative abundance of each indicator. The size of the circles represents the indicator value of each indicator. KO: *K. obovata*; AM: *A. marina*; SA: *S. alterniflora*; Mud: bare mudflat.

more N_2 was biologically fixed after the *S. alterniflora* invasion. In addition, the SA site had higher nitrification activity, but had lower anaerobic ammonium oxidation (anammox) and dissimilatory nitrate reduction to ammonium (DNRA, nitrate/nitrite ammonification) activities. The results of the FAPROTAX analysis imply that more NH_4^+ and NO_3^- were used for nitrification and denitrification at the SA site than at the three other mangrove sites.

4. Discussion

4.1. Effects of *S. alterniflora* invasion on soil biogeochemical properties

Plant species exert an important role in adjusting soil biogeochemical properties (Moreau et al., 2015). Our results showed that substrates content (e.g. SOM, TC, TOC, and TN) was significantly ($p < 0.05$) higher at the vegetated (AM, KO, and SA) sites (Table 1). A previous study in the Yangtze River estuary found that *S. alterniflora* had higher SOM, TC, and TN content than the native species *Scirpus mariqueter* (Cheng et al., 2008). *S. alterniflora* is a perennial C4 grass and is capable of a high degree of C fixation (Yang et al., 2016a). However, the concentration of SOM, TC, TOC, and TN at SA site were comparable to concentrations at mangrove sites (Table 1). This may be explained by the high productivity and the high turnover rates of SOM of the mangrove ecosystem (Biswas et al., 2007).

In addition, we found that the *S. alterniflora* invasion significantly ($p < 0.05$) increased soil NO_3^- content (Table 1), a finding that is consistent with the results reported by Peng et al. (2011). On one hand, the soil nitrate reductase and nitrite reductase activities were significantly ($p < 0.05$) enhanced after the *S. alterniflora* invasion, indicating that soil N availability and the N turnover rate were stimulated by the presence of *S. alterniflora* (Table 1) (Ehrenfeld, 2003; Yang et al., 2016a). On the other hand, the SA site is located near the sea and has relatively higher water content, suggesting that the water table is higher there. If so, this may account for the higher abundance of NO_3^- (Fig. 1, Table 1). The fact that *S. alterniflora* has a high level of fine root biomass may cause lower bulk density and trap more water in soil pore spaces during the ebb tide (Feng et al., 2017). This is significant because high water content and NO_3^- can favor soil denitrification (Yang and Silver, 2016). The soils dominated by mangrove species *K. obovata* and *A. marina* were neutral to slightly acidic, while a slightly alkaline soil was observed at the SA site (Table 1). These results agreed well with previous studies reporting that the *S. alterniflora* invasion was associated with significant increases in pH (Pan et al., 2016; Wang et al., 2016a). This may be due to an accumulation of NH_4^+ in the soil, since *S. alterniflora* prefers NO_3^- (Wang et al.,

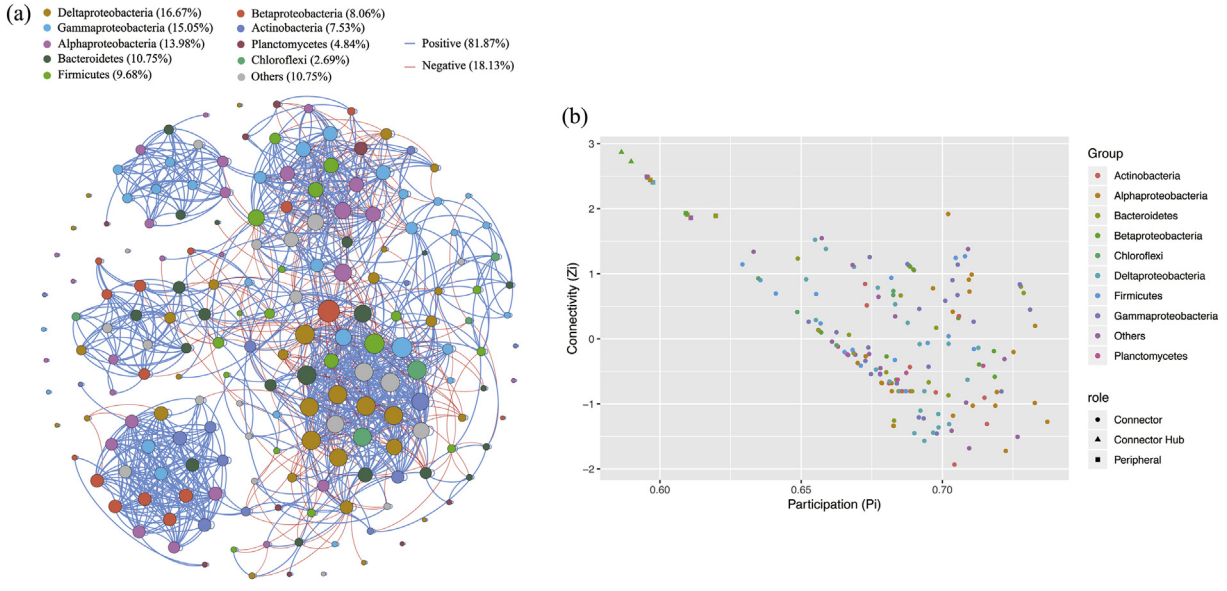

Fig. 7. A co-occurrence network features. The co-occurrence network of soil bacterial communities on the genus level with relative abundance>0.01% (a); the modularity roles of the nodes (b). The size of the circles in (a) represents the relative abundance of each genus.

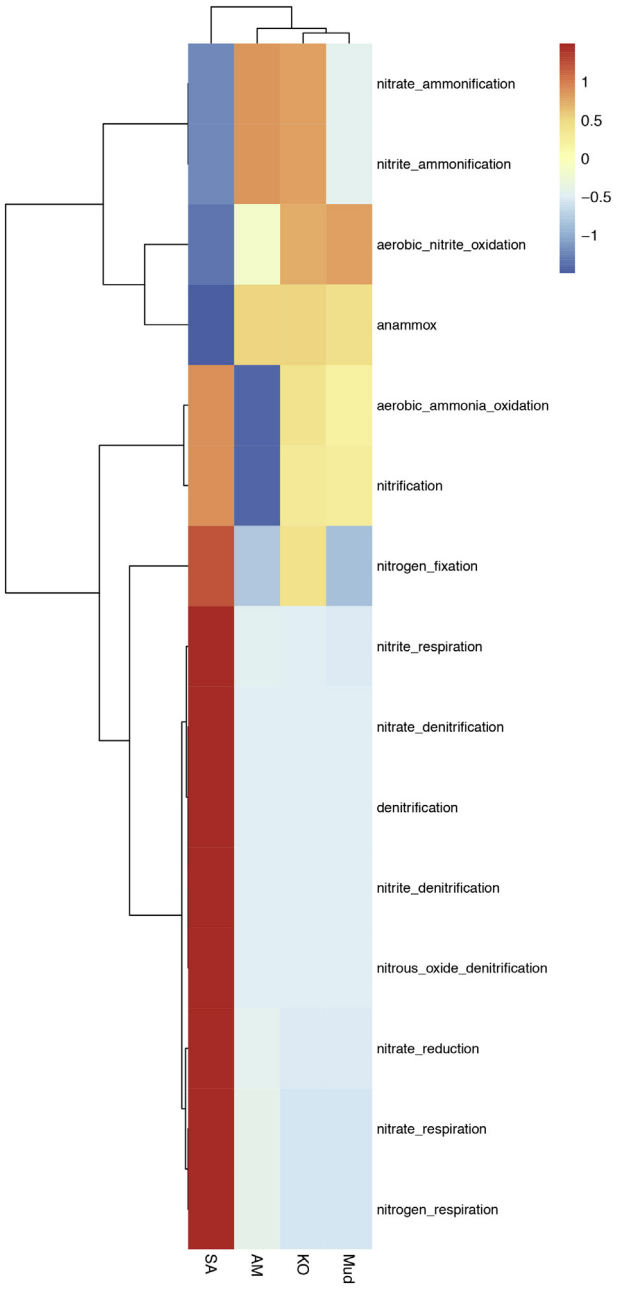

Fig. 8. Heatmap of the FAPROTAX analysis reveals the differences in soil nitrogen processes among sites. KO: *K. obovata*; AM: *A. marina*; SA: *S. alterniflora*; Mud: bare mudflat.

2016a). Moreover, the difference in root structure and exudation between mangrove species and *S. alterniflora* may also itself change the pH.

4.2. The S. alterniflora invasion altered the structure and composition of the soil bacterial community

Plant species shape the composition of the soil microorganism community, which mediates soil biogeochemical cycles (Knops et al., 2002). Our results confirmed that the *S. alterniflora* invasion into mangrove wetlands strongly modified the soil bacterial communities found there (Fig. 4, Table 3). This finding was consistent with other studies that reported significant changes in soil bacterial communities after the *S. alterniflora* invasion (Yang et al., 2016b; Liu et al., 2017b).

The indicator classes of bacteria *Chloroflexia*, *Alphaproteobacteria*, and *Bacilli* were strongly associated with the presence of *S. alterniflora* (Fig. 6). *Alphaproteobacteria* and *Bacilli* are characterized as denitrifiers, as they can form N_2O (Al-Attar and de Vries, 2015; Coyotzi et al., 2017). These results implied that the denitrifier community was changed after the *S. alterniflora* invasion, as reported by Bai et al. (2013) and Zhang et al. (2013a). The presence of these taxa may influence other bacteria via the network interactions, resulting in a significant change in bacterial community composition (Banerjee et al., 2018). Shifts in soil bacterial communities following the *S. alterniflora* invasion induced a significantly decrease in bacteria α-diversity; this diversity is strongly associated with multiple ecosystem functions (Wagg et al., 2014) (Table 2). Moreover, a previous study found that the Shannon diversity in the *S. alterniflora* rhizosphere was lower than the rhizosphere of *Phragmites communis* (Chen et al., 2012b).

Soil microorganisms closely communicated with their ambient environment. Plant species can affect soil microbial community in multiple ways, including via litter input and root exudation (Lange et al., 2015; Sasse et al., 2018). *S. alterniflora* has a highly developed root system and high photosynthesis capacity (Yang et al., 2016a). Different plant traits may increase habitats diversification, and this diversification is likely to affect the distribution of specific taxa (Liu et al., 2017b).

On top of being affected by the host plant, the microbial community is also strongly influenced by abiotic factors (Guevara et al., 2014). In our study, RDA results suggested that SOM and pH were the most important environmental factors regulating the bacterial community (Fig. 5). *S. alterniflora* may provide more available substrates for the growth of microorganisms via litter and root residual input (Yang et al., 2016c) (Table 1). High SOM accumulation may further stimulate soil microbial activity and enhance N turnover in *S. alterniflora* soil (Cheng et al., 2008). In addition, a previous study demonstrated that soil denitrifiers show high sensitivity to SOM accumulation (Fortunato et al., 2009). In addition, high SOM levels can increase water retention and can provide carbon and energy sources for microbial growth (Flores-Mireles et al., 2007).

pH is another key factor that has been found to regulate soil bacterial communities (Kotsyurbenko et al., 2007; Xia et al., 2015; Zhang et al., 2013a) (Fig. 5). There are two plausible interpretations that explain the important role of pH in shaping the bacterial community. Firstly, pH levels may select for species with compatible growth strategies (Feng et al., 2018). This is important because optimal bacterial growth occurs only within a narrow pH range (Ramirez et al., 2010); in this study, our results show that many species are sensitive to pH (Supporting Information Fig. 2). Moreover, varying pH also has a strong effect on the growth and proliferation of soil microorganisms (Rastogi et al., 2002). Secondly, pH has been found to be strongly correlated with soil nutrient availability (Anderson and Joergensen, 1997). Acidic soil has been found to depress the SOM decomposition and C mineralization (Anderson and Joergensen, 1997) and inhibit soil enzyme activity (Kiese and Butterbach-Bahl, 2002).

4.3. The S. alterniflora invasion stimulates soil denitrification and increases soil N_2O emissions

Denitrification and nitrification are strong determinants of soil N_2O emissions. However, in tropical and subtropical mangrove ecosystems, soil denitrification has been regarded as the main source of N_2O (Chiu et al., 2004; Fernandes et al., 2010). Although we did not investigate potential nitrification in the soil, our FAPROTAX analysis showed that the *S. alterniflora* invasion enhanced soil nitrification, which may contribute—at least in part—to soil N_2O emission levels (Fig. 8). Other studies have demonstrated that nitrifier bacterial growth and nitrification activity are promoted by *S. alterniflora* (Wang et al., 2015). However, FAPROTAX analysis is based on cultured prokaryotes, and therefore these results should be further verified in future studies.

The *S. alterniflora* invasion significantly ($p < 0.05$) enhanced the soil potential denitrification rate in mangrove wetland (Fig. 3). This is significant because the soil denitrification rate varies greatly among plant species (Fernandes et al., 2016). *S. alterniflora* has been shown to

stimulate denitrification by providing more substrates (Zhang et al., 2013b). Soil denitrification in coastal wetlands is generally limited by NO_3^- supply (Yang and Silver, 2016). However, in marine environments, the NO_3^- and NO_2^- are mainly involved in denitrification, rather than in other processes where nitrate is reduced to ammonium (Wankel et al., 2017). Similarly, our results showed that the *S. alterniflora* invasion decreased anammox and DNRA while increasing denitrification and nitrification, indicating that more NO_3^- was used for N_2O production at the SA site (Fig. 8). Moreover, bacteria involved in denitrification—such as *Alphaproteobacteria* and *Bacilli*—were strongly associated with *S. alterniflora*. Other studies also showed that the *S. alterniflora* invasion significantly increased both the abundance and diversity of denitrifiers (Bai et al., 2013; Zhang et al., 2013a). In addition, the *S. alterniflora* invasion was also found to promote the activity of nitrate reductase (Table 1). Taken together, these changes may account for the high level of soil denitrification after the *S. alterniflora* invasion.

In this study, we found that the *S. alterniflora* invasion significantly ($p < 0.05$) increased soil N_2O emissions in mangrove wetland (Fig. 2). Our results were consistent with other studies, one of which demonstrated that the *S. alterniflora* invasion caused an increase in soil N_2O emissions in coastal *P. australis* salt marshes (Zhang et al., 2013b). Along China's coast, the total area occupied by *S. alterniflora* was approximately 55, 181 ha in 2014 (Zhang et al., 2017). The average level of soil N_2O emissions at the *S. alterniflora* site was around 0.26 μmol m^{-2} h^{-1} (Fig. 2). If soil N_2O emissions from *S. alterniflora* habitats are similar along China's coastline, the total soil N_2O emissions from *S. alterniflora* habitats in China is approximately 0.06 Tg N_2O y^{-1}, and would accounted for 0.60% of the global N_2O emission total (9.6–10.8 Tg N_2O y^{-1}) (IPCC, 2013). As *S. alterniflora* is predicted to continue its rapid expansion in the future and to dominate in most coastal wetlands (Liu et al., 2017a; Zhang et al., 2017), the *S. alterniflora* invasion will continue to have an important impact on soil N dynamics and global climate change.

5. Conclusions

The *S. alterniflora* invasion in mangrove wetlands was found to significantly ($p < 0.05$) enhanced soil potential denitrification rates and soil N_2O emissions. In addition, the *S. alterniflora* invasion significantly ($p < 0.05$) decreased soil bacterial α-diversity and strongly altered soil bacterial community structure and composition. RDA showed that the soil bacterial community was largely shaped by surrounding environmental factors, such as SOM and pH. FAPROTAX analysis further suggested that the *S. alterniflora* invasion has had a great impact on soil N dynamics, stimulating denitrification and nitrification while depressing anammox and DNRA.

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Conflict of interests

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.10.277.

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